NEWBORN SCREENING NEWS

The California Newborn Screening Program **Winter 1999**

Changes to the Newborn Screening Program

In early 1999 the Newborn Screening (NBS) Program will be implementing several modifications in the screening program. These include:

- changes in determining the phenylketonuria (PKU) screen positives
- changes in reporting results
- the expansion of hemoglobin screening

Notification of the start date for implementing specific changes will occur via an insert in results mailer envelopes.

Changes in Determining Phenylketonuria (PKU) Screen Positives

For 18 years we have been using the phenylalanine concentration as an indicator for PKU. While this method is very accurate in identifying infants at risk for PKU, it yields a false positive rate of more than 80 newborns for every diagnosed case of PKU. In the past few years, we have been working toward using a phenylalanine/tyrosine (phe/tyr) ratio as an indicator of PKU which is as accurate as using phenylalanine concentration alone and is much more specific. Using a phe/tyr ratio we expect to see only 10-15 false positives for every diagnosed case of PKU. This is an enormous reduction in the false positive rate. We are confident that this method will correctly identify affected infants while dramatically decreasing the number of newborns who will need to be retested. This will reduce workload and other program costs as well as undue anxiety for the families of newborns and their pediatric care providers.

Rules for Determining PKU Screen Positive

1. The vast majority of patients will have their PKU screen results evaluated using the phe/tyr ratio. Ratios of 1.5 and above will be deemed positive and will require that a second specimen be collected.

2. Those specimens whose phenylalanine value is greater than or equal to 400 µmol/L will be deemed positive regardless of the ratio. This will be noted on the results mailer.

3. For those samples for which a tyrosine result cannot be obtained, we will base the PKU screen result on the phenylalanine value only and use a cut-off of greater than or equal to 200 µmol/L (which is the current cut-off). This will be noted on the results mailer.

Changes in Reporting Results

The NBS results mailers will be modified in the following manner:

1. **Actual values** – We will be reporting the actual values for all tests run. In the past the only numerical value reported was the phenylalanine result. Primary congenital hypothyroidism and galactosemia results were reported as "negative" or "positive." The new reports will include numerical results for all (non-hemoglobin) tests we perform.

2. **Complete information** – In keeping with CLIA standards, we will be reporting the actual test value, the unit of measurement the cut-off for positivity, and an interpretation for each test. We will be reporting all results using accepted international units.

PKU

The big change in Newborn Screening relates to PKU. In the past, we have reported phenylalanine results in mg/dl (milligrams per deciliter). When we begin using the phe/tyr ratio to determine PKU positives, we will compare µmol/L (micromoles per Liter) of phenylalanine to µmol/L of tyrosine. In addition to reporting the ratio, we will report the actual phenylalanine and tyrosine values in µmol/L. To ease the transition into this new reporting format, we are providing you with the conversion factor for converting the phenylalanine level in µmol/L to mg/dl:

Phenylalanine µmol/L x 0.01652 = phenylalanine mg/dl
Example: 200 µmol/L x 0.01652 = 3.3 mg/dl
(truncated to the tenths position)

Reporting of High Tyrosine Values

The California Newborn Screening Program is **not** screening for abnormalities of tyrosine metabolism. There are insufficient data regarding this group of metabolic disorders to easily and readily distinguish them from benign transient tyrosine elevations. However, actual tyrosine results will be provided on the NBS results mailer and values of 700 µmol/L or more will be highlighted as very unusual (approximately 4 each month are anticipated). Additional evaluation is up to the pediatric care provider.

The State recommends collecting a second specimen for all infants with an initial tyrosine value of 700 µmol/L or more and sending it to the State Genetic Disease Laboratory for testing, which is free of charge. The specimen should be collected approximately 5-7 days after supplementation with vitamin C and adjustment in protein intake. Infants with recall results of 700 µmol/L or greater should be referred to a metabolic specialist.

Benign transient tyrosine elevations occur frequently in premature infants with high protein intakes (>5 gm/kg of dietary intake) and/or inadequate vitamin C. Reducing protein intake to 3 gm/kg of dietary intake per day and supplementation with 50-100 mg per day of vitamin C is recommended. Such infants usually respond within two or three weeks to lower protein intake and vitamin C supplementation. In contrast, tyrosine concentrations in patients with metabolic disorders usually remain high and are commonly accompanied by development of other symptoms.

For more information on tyrosinemias consult a metabolic specialist. The Newborn Screening Coordinators can assist in identifying referral or consultation resources and/or a laboratory for testing tyrosine concentrations.

Reporting of Other Disorders

We will be reporting transferase results in enzyme units. Those specimens whose results are 40 enzyme units or less will be considered positive for the galactosemia screen. In these cases, we require that a heparinized whole blood specimen be sent to the Biochemical Genetics Laboratory at Childrens Hospital in Los Angeles. There is no known upper limit for the transferase value and physicians need not be concerned about high values.

TSH results will continue to be reported in mIU/L. Results greater than the highest standard will be reported as " > 250 mIU/L." Results below the limit of detection will be reported as " < 0.2 mIU/L."

Hemoglobin results will continue to be reported as a Hb pattern with an interpretation. The types of Hb are listed in order of their relative frequency, but we will not be reporting the percentages of each type of hemoglobin.

Expansion of Hemoglobin Screening

Clinically significant hemoglobinopathies currently identified by the NBS Program include sickle cell disease (sickle cell anemia, sickle hemoglobin C, sickle hemoglobin D, sickle hemoglobin E, and sickle Beta thalassemia), Beta^0 thalassemia, and hemoglobin E/Beta thalassemia. The program will be expanded to identify hemoglobin H (Hb H) disease including the more severe form of this disorder Hb H-Constant Spring disease. Additionally, a few cases of alpha thalassemia major will be identified if a NBS sample can be obtained. Because of the conservative cutoff for Hb H disease, a small number of alpha thalassemia traits will also be identified. *(See page 3 for a description of the different forms of alpha thalassemia.)*

Hemoglobin H is an abnormal hemoglobin found in adults with alpha thalassemia. It is a tetramer of beta globin chains (4β) formed when there are insufficient alpha (α) chains to make normal adult hemoglobin (2α, 2β). The fetus manufactures gamma (γ) chains rather than β chains, and the tetramer of γ chains that forms in alpha thalassemia is called hemoglobin Barts (4γ). During the newborn period Hb Barts, if present, can be detected. However, Hb Barts decreases with the normal decrease in gamma chain production and therefore, over time, it disappears and is replaced by Hb H.

Since fall of 1996 we have been conducting a pilot Hb H screening project. The goals of this project were to determine how reliably hemoglobin Barts can be detected using our high performance liquid chromatography (HPLC) screening method and to determine the feasibility of establishing a reasonable cutoff for Hb H disease. We have learned that a peak at the origin of the hemoglobin pattern correlates reliably with hemoglobin Barts. We have arrived at a presumed Barts cutoff of 25% in this "fast window" of the chromatogram. This appears to detect most of the cases of Hb H disease while minimizing the number of false positives for disease, which are actually two gene deletion cases, a clinically benign carrier condition known as alpha thalassemia trait.

We will report all cases at and above the 25% cutoff. They will be followed as are other hemoglobin disorders. Confirmatory testing including DNA will be provided and referral to a California Children's Services (CCS) Hematology Center is strongly recommended. As a result of the pilot project, we anticipate that approximately one-third of the Hb H cases will be the more clinically serious Hb H/Constant Spring disease. Newborn Screening Coordinators at Area Genetic Centers

across the State will assist with referrals to CCS Hematology Centers.

The presence of presumed hemoglobin Barts below the cutoff will ***not*** be noted on the NBS results mailer, nor will follow-up be provided. Most infants with alpha thalassemia trait will have fast hemoglobin below the 25% cutoff. Furthermore, while samples were tested as low as 15% during the pilot project without identifying a presumed case of Hb H disease between the 15 – 25% range, we have not eliminated the possibility that cases of Hb H disease could have fast hemoglobin percentages below the 25% cutoff. **As with all population based screening, it is possible that a newborn with Hb H disease could have a percentage of "fast" hemoglobin below the cut-off and therefore not be reported.**

Alpha Thalassemia

Thalassemias are a group of conditions which have in common the inability to produce sufficient quantities of globin chains necessary for hemoglobin synthesis. The most common types of thalassemias are alpha and beta, which are named according to the type of chain affected. The focus of this discussion is alpha thalassemia.

There are four genes which code for alpha chain production. Alpha thalassemia results when one or more of these genes are not working. The number and location of the non-working genes determines the type of alpha thalassemia an individual has.

Types of Alpha Thalassemia:

Alpha thalassemia "silent carrier": Deletion of one alpha globin gene. This is clinically benign, usually with no clinical manifestations.

Alpha thalassemia trait (also called alpha thalassemia minor): Deletion of two alpha globin genes. This trait is also clinically benign, with the clinical manifestations being microcytosis and mild, if any, anemia. This is often confused with iron deficiency anemia. Unless the individual also has iron deficiency anemia, iron supplementation is usually not recommended.

Hemoglobin H (Hb H) disease: Deletion of three alpha globin genes. The clinical complications associated with Hb H disease are variable. This generally results in mild to moderate anemia, and is often associated with microcytosis, hypochromia, and red cell fragmentation.

Hemoglobin H (Hb H)-Constant Spring disease: Deletion of two alpha globin genes and a point mutation of a third. This is usually a more severe form of Hb H disease, usually with a moderate to severe clinical course. Complications include the development of splenomegaly and cholelithiasis. Some individuals may require intermittent to chronic transfusions. Clinical symptoms for both forms of Hb H disease that can begin at birth include pallor and jaundice. In addition, severe anemia can be caused by certain types of medications (including aspirin, sulfa drugs, some antibacterials) as well as fava beans and moth balls.

Alpha thalassemia major (Hydrops Fetalis): Deletion of all four alpha globin genes. No alpha chains, which are necessary for the formation of fetal hemoglobin, are produced. Death usually occurs in utero or early infancy. For infants who survive the neonatal period, treatment consists of ongoing transfusions.

When three or more alpha globin genes malfunction, there is an excess of beta globin chains. The excess chains create unstable tetramers called hemoglobin H. These tetramers eventually precipitate in the red blood cells, causing membrane damage and premature destruction of the cells producing a chronic hemolytic anemia. During the newborn period, when gamma globin production is still high and beta globin production is low, the gamma chains form the unstable tetramers identified as hemoglobin Barts. It is the identification of large amounts of Hb Barts that leads us to presume the infant may have Hb H disease. DNA testing is necessary to make the final diagnosis.

California Children's Services Hematology Centers

Sutter Memorial Hospital, Sacramento, 916/733-1757
UC Davis Medical Center, Sacramento, 916/734-2782
UC San Francisco, Med. Ctr., San Francisco, 415/476-1000
Children's Hospital of Northern CA, Oakland, 510/428-3372
No. California Kaiser Permanente, Oakland, 510/596-6592
Valley Children's Hospital, Madera, 559/229-3409
Saint Agnes Medical Center, Fresno, 559/449-5378
City of Hope Medical Center, Duarte, 818/359-8111
Orthopedic Hospital, Los Angeles, 213/742-1000
UC Los Angeles Medical Center, Los Angeles, 310/825-6708
Childrens Hospital of Los Angeles, Los Angeles, 323/669-2121
Cedars Sinai Medical Center, Los Angeles, 310/855-2941
Kaiser Foundation Hospital, Los Angeles, 323/783-4011
Harbor-UCLA Medical Center, Torrance 310/222-4154
Long Beach Memorial Med. Ctr., Long Beach 310/933-2000
Loma Linda Univ. Medical Center, Loma Linda, 909/824-0800
San Bernardino Co. Med. Ctr., San Bernardino, 909/387-7705
UC Irvine Medical Center, Orange, 714/456-8411
Children's Hospital of Orange Co., Orange, 714/532-8636
UC San Diego, Medical Center, San Diego, 619/543-8273
Children's Hospital & Health Ctr., San Diego, CA 619/576-1700

Should Newborn Screening Specimens be Collected from IV Lines (Peripheral, Umbilical or Central)?

No one wants to "stick" a baby when it is unnecessary. It is understandable that lab or nursing personnel may prefer to collect NBS blood specimens from lines, when present, rather than from heelsticks. However, the use of blood drawn from lines for newborn screening can produce spurious results, leading to unnecessary re-screening, or worse, missed cases.

We have found differences in analyte concentration of blood specimens collected by different methods (heelstick, dorsal vein, capillary tube)[1]. The test cutoffs utilized are based on heelstick specimen values. For this reason, we do not allow specimen collection by the other two methods. While we have not studied the analyte concentrations in IV specimens, there is no question that the odds of specimen contamination are great, given the number of substances going through IV lines. Furthermore, some of the analytes are known to cling to the sides of the tubing, which could skew the test results.

For the above reasons, the Genetic Disease Branch discourages the use of IV lines to obtain NBS specimens. The fact that a baby has a line should not preclude the utilization of the heelstick method. However, there are some clinical conditions for which heelsticks would be contraindicated, e.g., anemia or very tiny and poorly perfused feet. In these situations, a line specimen is preferable to not screening at all.

If a specimen is to be drawn from an IV line, it is imperative that the line be thoroughly cleared first. Those responsible for collecting specimens should strictly adhere to their facility's protocol for accomplishing this.

Our data shows that NICU babies have a higher incidence of the screened disorders, and yet are at greater risk of not being screened at all. Whichever method is used, what is most important is that these babies are screened.

1 Lorey FW, Cunningham GC. Effect of specimen collection method on newborn screening for PKU. Screening 1994; 3:57-65

For patient education materials, contact the Newborn Screening Program, GeneHELP Resource Center at (510) 540-2534.

Senate Bill 537

Senate Bill 537, authored by Senator Greene, became law on September 29, 1998. This bill requires the Department to establish a program to provide extended newborn screening services. This may include, but need not be limited to: congenital adrenal hyperplasia, cystic fibrosis, and biotinidase deficiency. Additional testing for medium chain Acyl-CoA dehydrogenase deficiency (MCADD) and other fatty acid oxidation disorders will be part of a demonstration project at additional cost. The Department must report to the Legislature regarding the progress of the program by June 30, 2000. The report shall include the costs for screening, follow-up, and treatment as compared to the costs and morbidity averted for each condition tested for in the program. As soon as funds are approved the Department will begin the process of implementing the bill.

Newborn Screening Coordinators at Area Genetic Centers (AGCs)

UC San Francisco	(415) 476-5048
UC Davis	(530) 754-5400
Children's Hospital, Oakland	(510) 428-3127
Stanford University	(650) 723-7987
Valley Children's Hospital	(559) 353-6416
Loma Linda University	(909) 558-4191
UCLA Medical Center	(310) 825-9719
Childrens Hospital, Los Angeles	(323) 669-2226
Harbor/UCLA Medical Center	(310) 222-3751
UC Irvine Medical Center	(714) 456-6878
San Diego-Imperial Counties Developmental Services	(619) 576-2975
Kaiser Permanente, Northern CA	(510) 596-6192
Kaiser Permanente, Southern CA	(626) 564-3325

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